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HOST DEFENSE AGAINST OPPORTUNIST MICROORGANISMS FOLLOWING TRAUMA

Annual Report

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Studies were conducted to determine the effects of parenteral therapy with indomethacin, ibuprofen, and piroxicam on key immunologic and hematologic alterations induced by thermal injury in a guinea pig model and on survival following experimental burn wound infection with Pseudomonas aeruginosa and Proteus mirabilis. Drugs (10-20 mg/kg) or placebo were administered intramuscularly to thermally injured guinea pigs at 3 hr postburn and then daily for nine days postburn. All three drugs inhibited production of prostaglandin $F_{1\alpha}$ and thromboxane B_2 in wound fluid and concomitantly restored the bactericidal activity of polymorphonuclear leukocytes (PMNs) against P. aeruginosa to normal. Indomethacin increased the proliferative response of splenic lymphocytes to concanavalin A at a dose of 10 mg/kg but not 20 mg/kg, and ibuprofen and piroxicam had no effect on this response. None of the drugs affected the extent of systemic complement consumption, thrombocytopenia, leukocytosis, or leukopenia in the injured animals,
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or survival following burn wound infection with P. aeruginosa or P. mirabilis. These results suggest that the PMN bactericidal defect induced by thermal injury is preventable or reversible, and that the mechanisms responsible for this defect are inhibitable by nonsteroidal anti-inflammatory drugs. Failure of these drugs to correct additional major alterations of host defense (i.e., complement consumption and the impairment of cell mediated immunity) may explain why they did not increase survival following burn wound infection.

FOREWORD

In conducting the research described in this report, the investigators adhered to the Guide for the Care and Use of Laboratory Animals, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 86-23, Revised 1985). Citations of commercial organizations and trade names in this report do not constitute an offical Department of the Army endorsement or approval of the products or services of these organizations.

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BODY OF REPORT

Statement of the Problem under Study

Accidental injury is the leading cause of premature mortality in the United States [1]. Approximately 40% of the deaths occurring before age 65 result from accidental injuries. The number of subjects admitted for inpatient hospital treatment of burns is estimated to be in the range of 60,000 to 90,000 per year [2]. Thermal injury profoundly depresses the defense mechanisms of the body which under normal conditions protect against microbial infection. This results in a marked increase in the susceptibility of thermally injured patients to bacterial infection. Bacteria from the immediate environment colonize the burn wounds and frequently cause life-threatening infections. The advent of topical antimicrobial therapy, the development of more effective topical and systemic antibiotics, and early excision and grafting of the burn wound have decreased the mortality directly related to bacterial infections in thermally injured patients [3]. Further progress in reducing burn mortality due to bacterial infection is dependent on understanding the fundamental mechanisms responsible for the depression of host defense associated with thermal injury.

We have used a well characterized animal model to investigate the role of various inflammatory mediators in key cellular alterations of host defense induced by thermal injury under controlled conditions. Our studies have demonstrated a temporal association between production of arachidonic acid metabolites of the cyclooxygenase pathway, consumption of complement, and depression in the bactericidal activity of polymorphonuclear leukocytes (PMNs) in the injured animals [4]. In the present study, this association was further investigated using parenteral therapy with nonsteroidal anti-

inflammatory drugs (NSAIDs) to inhibit prostaglandin and thromboxane production and by determining the effects on complement consumption and the bactericidal activity of PMNs. Effects of the NSAIDs on the lymphoproliferative response to T cell mitogen, on various hematologic changes induced by thermal injury, and on survival following experimental burn wound infection with Pseudomonas aeruginosa and Proteus mirabilis were also investigated. Since certain NSAIDs have discrete inhibitory effects on PMNs that occur independently of the classic inhibitory action of these drugs on the cyclooxygenase enzyme [5], three chemically unrelated NSAIDs were used in our study, i.e. indomethacin, ibuprofen, and piroxicam. The assumption was made that if similar effects were observed with all three drugs, then these effects could be attributed to inhibition of cyclooxgenase or to another therapeutic action shared by these drugs.

Methods

Animals. Male and female Hartley guinea pigs weighing 300 to 350 g were purchased from Murphy Breeding Laboratories, Inc., Plainfield, IN. The animals were housed in separate cages and adapted to the new environment for four to seven days. The animals were fed guinea pig chow ad libitum.

Experimental thermal injury. Full thickness scald burns covering thirty percent of the total body surface were applied under anesthesia as previously described [4]. Sham treated animals were anesthetized and then immersed in tepid water. Fifteen ml of lactated Ringer's solution (Abbott Laboratories, North Chicago, IL) was administered intraperitoneally to all animals before and at 1.5 hr after injury or sham treatment. In some experiments, stainless steel mesh wound chambers were implanted under the skin on the dorsum, and the animals were burned or sham injured seven days later [6]. Survival of burned animals during 21 days postburn was approxi-

mately 75%.

Preparation and administration of drugs and placebo. The salt form of indomethacin (Merck, Sharpe, & Dohme, Westpoint, PA) was dissolved in 0.01 M phosphate buffered saline, pH 7.4 (PBS). Piroxicam (Pfizer, Westpoint, PA) was solubilized in 0.2 M sodium phosphate buffer, pH 7.4, at a concentration of 7.5 mg/ml by dropwise addition of 2 N sodium hydroxide to pH 8.5; the solution was then titrated back to pH 7.4 with 6 N hydrochloric acid and used immediately. Ibuprofen (50 mg/ml; Upjohn Co., Kalamazoo, MI) was diluted in PBS to 3.75 mg/ml. The drugs were prepared fresh daily and administered intramuscularly at 3 hr postburn and then daily for 9 days postburn. Indomethacin and piroxicam were administered once daily in doses of 10 mg/kg and 15 mg/kg respectively. In some experiments, doses of 5 or 20 mg/kg/day of indomethacin were also used. Ibuprofen was administered twice daily in a divided dose of 7.5 mg/kg. PBS served as placebo and was administered once or twice daily depending on the drug regimen in the same volume as the drugs.

Collection and handling of specimens. Blood was drawn by cardiac puncture under anesthesia into plastic syringes. Blood was heparinized for preparation of PMNs (50 units/ml of blood). Blood for complete blood counts and platelet counts was added to microtainer capillary whole blood collectors containing ethylenediaminetetraacetate (Becton Dickinson & Co., Rutherford, NJ). For preparation of serum, blood was clotted in glass tubes at room temperature and then stored at 4°C for up to 4 hr. The tubes were centrifuged at 800 g for 10 min at 4°C. Serum was removed and stored in small aliquots at -70°C. Spleens were removed aseptically, and single cell suspensions were prepared by gentle teasing with glass microscope slides into RPMI 1640 (M.A. Bioproducts, Walkerville, MD) containing 0.35% HEPES (Sigma Chemical Co., St. Louis, MO), 0.1% gentamicin (Schering Corp.,

Kenilworth, NJ), and 5% heat-inactivated fetal calf serum (KC Biological, Lenexa, KS); this medium will be referred to hereafter as RPMI medium. Wound fluid was immediately supplemented with 5 μM meclofenamate (Warner-Lambert Co., Ann Arbor, MI) to prevent artifactual biosynthesis of arachidonate metabolites in vitro and centrifuged at 1,000 g for 10 min at 4°C. Supernatants were removed and stored at -70°C. Animals were euthanized after collection of specimens.

Measurement of arachidonic acid metabolites. Six-keto prostaglandin (PG) $F_{1\alpha}$ and thromboxane B_2 were measured in wound fluid using radioimmunoassay kits from Advanced Magnetics Inc., Boston, MA. The method outlined by the manufacturer was followed, with the addition that three twofold dilutions of each sample were tested and sample blanks were included. Results from two dilutions on the linear part of the curve were averaged; these results varied by less than 20%. Spiking experiments were also performed using two samples of wound fluid from sham injured and burned animals obtained preburn and at one day postburn. Samples were supplemented with 10,000 pg/ml of standard. Analysis of these samples showed the expected increase.

PMN bactericidal assays. Two methods were used for measurement of the bactericidal activity of PMNs. For both methods, PMNs were prepared from heparinized blood by dextran sedimentation followed by centrifugation through lymphocyte separation medium (Organon Teknika Corp., Durham, NC) and hypotonic lysis of contaminating erythrocytes [7]. PMNs were suspended in Hank's balanced salt solution containing 0.1% gelatin (Difco Laboratories, Detroit, MI) (HBG). Suspensions contained 78% to 92% PMNs. For both methods, a clinical isolate of P. aeruginosa, strain Wk, was grown for 4 hr at 37°C in trypticase soy broth (BBL, Cockeysville, MD). The bacteria were washed and resuspended in PBS.

In the first method, PMN bactericidal activity was measured using an acridine orange staining technique [8]. One hundred µl of bacteria (2.0 x 10^7 cfu), 200 µl of leukocyte suspension (2.0 x 10^6 cells), and 100 µl of pooled normal guinea pig serum were mixed together, and 300 µl was layered on coverslips (22 x 40 mm; no. 1 thickness). The coverslips were incubated in a moist chamber for 30 min at 37°C in 5% CO_2 and then washed with warmed Hank's balanced salt solution. The coverslips were stained for 1 min with 0.015% acridine orange (Fisher Scientific Co., Fair Lawn, NJ) in Hank's balanced salt solution, washed with the latter solution, and mounted as described previously. Dead (red) and live (green) PMN-associated bacteria were counted using a Zeiss fluorescent microscope (Carl Zeiss, Inc., Thornwood, NY); 50 PMNs were viewed. Percent killing was calculated by dividing the number of dead bacteria by the total bacteria and multiplying by 100. The percent of PMNs with associated bacteria was determined by dividing the number of PMNs with associated bacteria by 50 and multiplying by 100. The number of bacteria per PMN was calculated by dividing the total number of PMN-associated bacteria by 50.

In the second method, PMN bactericidal activity was measured by enumerating total surviving bacteria by colony counting [9]. Bacteria (5.0 x 10^8 cfu/ml) were first incubated for 15 min at 37°C with 50% pooled normal guinea pig serum (vol/vol). The opsonized bacteria were washed once and resuspended in HBG. Opsonized bacteria (1.0 x 10^7 cfu/ml) and 5.0 x 10^6 leukocytes/ml were rotated for 60 min at 37°C. Fifty µl aliquots were removed before and after incubation, serially diluted in distilled water, and the dilutions were plated on MacConkey agar (Difco Laboratories). Percent killing was calculated by the formula (a-b)/a x 100, where a and b were equal to surviving cfu/ml before and after incubation respectively.

Complement determinations. A minor modification of the method of Kaneko

et al. [10] was used to measure lysis of rabbit erythrocytes mediated by the alternative complement pathway in guinea pig sera. Two hundred and fifty μl of twofold dilutions of test serum were prepared in isotonic veronal buffered saline, pH 7.4, containing 8 mM ethylene glycol tetraacetic acid (Sigma Chemical Co.) and 4 mM magnesium chloride (EGTA-Mg). Fifty μl containing 1.5×10^7 rabbit erythrocytes (Colorado Serum Co., Denver, CO) in EGTA-Mg was added, and the reactants were incubated for 60 min at 37°C. The reaction was stopped by addition of 1.7 ml of cold 0.15 M sodium chloride, pH 7.0. Erythrocytes were deposited by centrifugation, and hemoglobin in the supernatants was measured spectrophotometrically at 415 nm. Controls included a reagent blank in which EGTA-Mg was substituted for the serum and a water lysate (2 ml) of 1.5×10^7 rabbit erythrocytes. Results were expressed as CH_{50} units/ml.

Immunochemical concentrations of C3 in guinea pig sera were determined by radial immunodiffusion [11]. Monospecific antiserum to guinea pig C3 was raised in a goat by repeated subcutaneous injections of guinea pig C3 (Cordis Laboratories, Miami, FL) in Freund's complete adjuvant (Difco Laboratories). A pool of normal guinea pig serum was used as the reference standard. The C3 concentration in this reference serum was determined using purified guinea pig C3 as the standard. This material was prepared by the method of Thomas and Tack [12] and was homogeneous as assessed by SDS polyacrylamide gel electrophoresis [13] and double immunodiffusion using antisera directed against guinea pig C3 and whole guinea pig serum (Colorado Serum Co.).

Measurement of lymphoproliferative responses to mitogen. Spleen cell suspensions were washed twice and adjusted to 3.0×10^6 cells/ml in RPMI medium. Two hundred μl aliquots of the cell suspensions were dispensed into Falcon microtest III tissue culture plates (Becton Dickinson & Co.,

Lincoln Park, NJ). Fifty µl of RPMI medium containing 1 µg of concanavalin A (Con A; Sigma Chemical Co.) or no mitogen was added, and the plates were incubated at 37°C for 48 hr in 5% CO_2. Fifty µl of RPMI medium containing 0.2 µCi of [^{3}H]thymidine (6.7 Ci/mmol; New England Nuclear, Boston, MA) was added, and the incubation was continued for 18 hr. Cells were collected using a Mash II harvester (M.A. Bioproducts), and radioactivity was counted in a Beckman LS 7000 liquid scintillation counter (Beckman Instruments, Palo Alto, CA); standard methods were used. Determinations were performed in triplicate. Viability was measured in duplicate in separate plates containing cells cultured without mitogen. Fluorescent microscopy of fluorescein diacetate/ethidium bromide stained cells was used to assess viability [14].

Complete blood counts and platelet counts. These determinations were performed in the Central Hematology Laboratory of The Christ Hospital, Cincinnati, OH, using a Coulter S+V (Coulter Electronics Inc., Hialeah, FL) or an Ortho ELT-800 hematology analyzer (Ortho Diagnostic Systems Inc., Westwood, MA).

Experimental burn wound infection. *P. aeruginosa* 12-4-4 was obtained from Dr. Albert McManus, Brooke Army Medical Center, Fort Sam Houston, TX. A blood culture isolate of *P. mirabilis* from a thermally injured patient at the University of Cincinnati Medical Center, Cincinnati, OH was also used. The bacterial strains were grown for 4 hr at 37°C in trypticase soy broth. The lethal dose $(LD)_{50}$ was spread over the surface of the burn wounds with a sterile bent glass rod at 1.5 hr postburn; the LD_{50} was 1.0×10^5 cfu for *P. aeruginosa* 12-4-4 and 7.5×10^3 for the clinical isolate of *P. mirabilis*. In some experiments, the bacterial challenge was administered at one day postburn. Survival was assessed for seven to nine days postburn. Preliminary bacteriologic and histologic studies documented that both

bacterial strains caused invasive burn wound sepsis in thermally injured guinea pigs.

Statistical methods. Significant differences between data were determined by analysis of variance [15].

Results

Potency of the NSAIDs as cyclooxygenase inhibitors in the injury model. Preliminary experiments were performed to insure that parenteral therapy with the doses of NSAIDs used in our experiments effectively inhibited prostaglandin and thromboxane production in the immediate area of thermal injury. Wound chambers were implanted under the area of skin that was subsequently burned or sham injured, and fluid was collected from the wound chamber (wound fluid) immediately prior to injury and then daily for four days postburn. Levels of 6-keto $PGF_{1\alpha}$ and thromboxane B_2 were measured in wound fluid harvested from injured animals treated with NSAIDs or placebo and from sham injured animals. Levels of 6-keto $PGF_{1\alpha}$ and thromboxane B_2 were significantly lower in wound fluid from injured animals treated with indomethacin, ibuprofen, or piroxicam than in injured animals treated with placebo from two through four days postburn ($P<0.05$; Figure 1). These results documented that the drug regimens used in our experiments inhibited prostaglandin and thromboxane production at the site of thermal injury.

Effects of the NSAIDs on the bactericidal activity of PMNs. In initial experiments, bactericidal activity of PMNs against P. aeruginosa was measured using an acridine orange staining technique. Each drug was analyzed in a separate experiment, each having its own placebo and normal control groups. Placebo was administered once or twice daily in accordance with the drug regimen. Two animals from the drug, placebo, and normal control groups were sacrificed at various time intervals during nine days

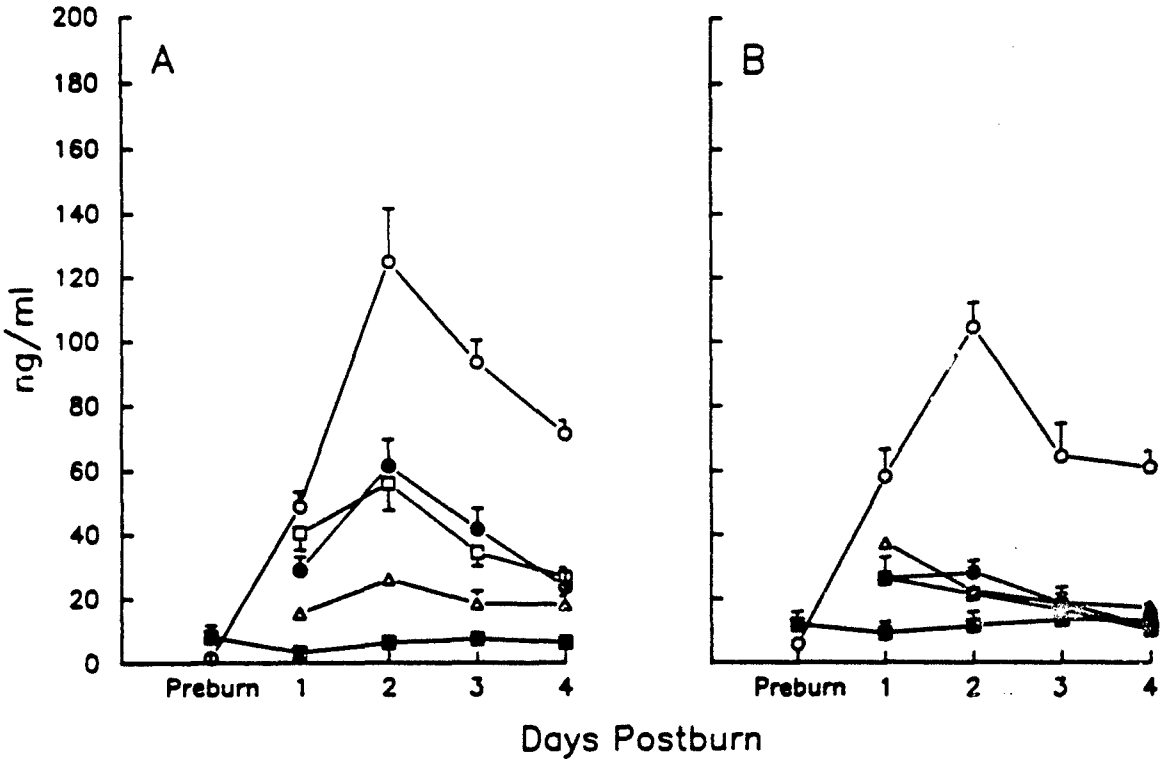

Figure 1. Levels of 6-keto $PGF_{1\alpha}$ (A) and thromboxane B_2 (B) in wound fluid from thermally injured animals treated with 10 mg/kg indomethacin (●), 15 mg/kg ibuprofen (△), 15 mg/kg piroxicam (□), or placebo (○), and from sham-injured animals (■). Data are expressed as mean ± SEM. There were four or five animals per group.

postburn. PMNs from animals within each group were pooled and assayed with pooled normal guinea pig serum. As reported previously [4], bactericidal activity of PMNs from injured animals was significantly reduced during nine days postburn as compared with PMNs from normal animals ($P<0.05$; Figure 2). Treatment of the injured animals with indomethacin, ibuprofen, or piroxicam restored the bactericidal activity of PMNs to normal. This effect was manifested at one day postburn and continued for nine days postburn. The percent of PMNs with associated bacteria and the number of bacteria per PMN, two parameters reflecting phagocytosis, were similar in PMNs from normal animals and injured animals treated with drugs or placebo; mean values ranged from 98% to 100% PMNs with associated bacteria and 4.9 to 6.6 bacteria per PMN. These results suggested that the NSAIDs enhanced the process of killing without affecting phagocytosis.

Bactericidal activity of PMNs was also measured using a second technique in which P. aeruginosa opsonized with pooled normal guinea pig serum were incubated with PMNs in suspension, and total surviving bacteria were enumerated by colony counting. In these experiments, PMNs were harvested on the first and second days postburn, and the kinetics of bactericidal activity were determined. These experiments confirmed that parenteral therapy of injured animals with indomethacin, ibuprofen, or piroxicam restored the bactericidal activity of PMNs to normal (Figure 3).

Effects of the NSAIDs on complement. Recent studies have suggested that systemic complement consumption resulting from thermal injury leads to functional inactivation of PMNs [16]. Since NSAIDs are capable of inhibiting complement consumption [17], experiments were conducted to determine if NSAIDs enhanced the bactericidal activity of PMNs in injured animals by this mechanism. Since thermal injury activates complement primarily via the alternative pathway [18], hemolytic activity in sera selectively me-

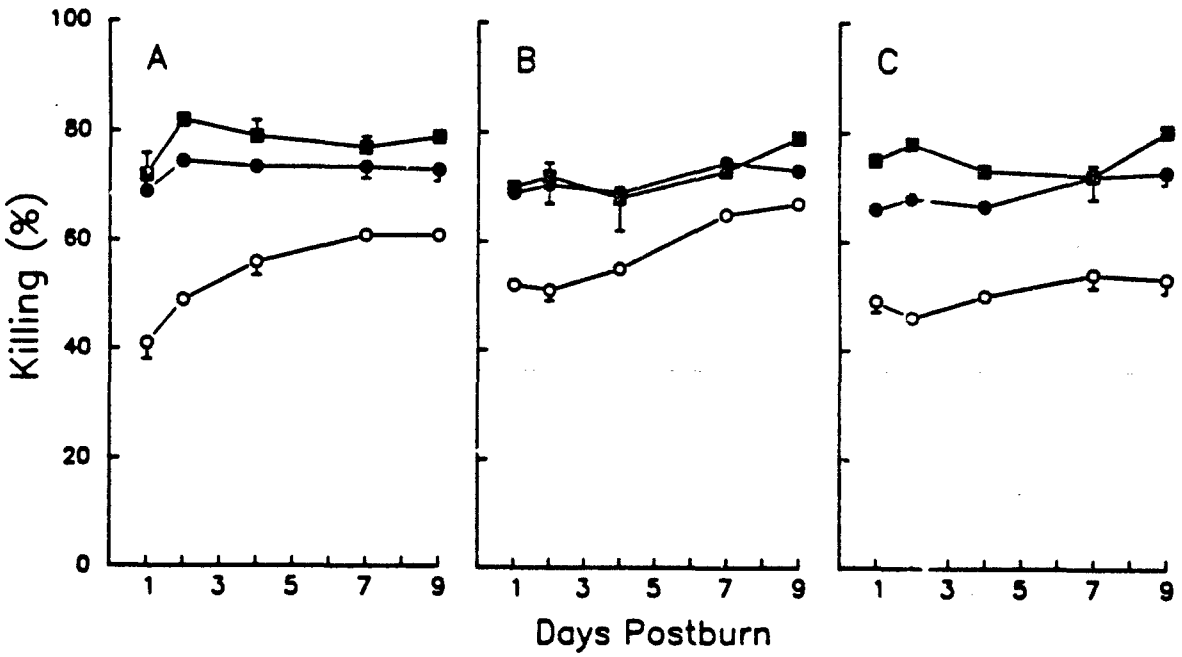

Figure 2. Bactericidal activity of PMNs from thermally injured animals treated with NSAIDs (●) or placebo (○) and from normal animals (■). Ten mg/kg indomethacin (A), 15 mg/kg ibuprofen (B), and 15 mg/kg piroxicam (C) were used in separate experiments, each having its own placebo and normal control groups. PMN bactericidal activity was measured using an acridine orange staining technique. Data are expressed as mean ± SEM of duplicate determinations.

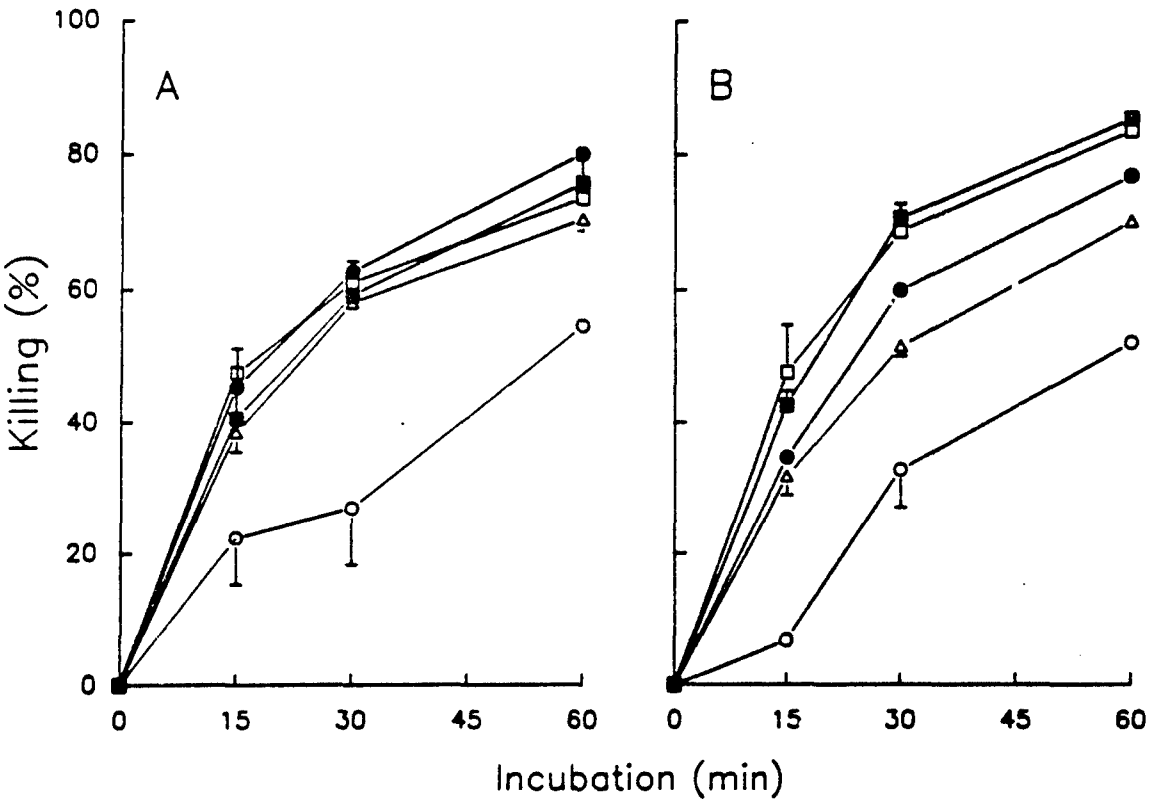

Figure 3. Kinetics of bactericidal activity of PMNs from thermally injured animals treated with 10 mg/kg indomethacin (●), 15 mg/kg ibuprofen (△), 15 mg/kg piroxicam (□), or placebo (○), and from normal animals (■). PMNs were harvested at one day postburn (A) or two days postburn (B). PMN bactericidal activity was measured by enumerating total surviving bacteria by colony counting. Data are expressed as mean ± SEM of duplicate determinations.

diated by this pathway was measured in our study. Immunochemical serum C3 concentrations were also determined.

Hemolytic activity and C3 concentrations were significantly reduced in sera from injured animals early after injury (one or two days postburn) as compared with sera from normal animals ($P<0.05$; Figures 4 and 5). These measurements increased to normal or supranormal levels in the injured animals by nine days postburn. Hemolytic activity was maximally increased in animals receiving two injections of drug (ibuprofen) or placebo each day; these animals may have experienced an exacerbated acute phase response related to inflammation at the injection sites. The C3 level at two days postburn was significantly higher in injured animals treated with piroxicam than in injured animals treated with placebo ($P=0.006$). However, no other significant differences in C3 concentration or hemolytic activity were observed in sera from injured animals treated with indomethacin, ibuprofen, or piroxicam as compared with placebo. These results tended to rule out the hypothesis that the NSAIDs enhanced PMN bactericidal activity by inhibiting complement consumption.

Effects of the NSAIDs on the lymphoproliferative response to T cell mitogen. The effects of the NSAIDs on the proliferative response of splenic lymphocytes to concanavalin A were also investigated. Since our previous studies had shown that lymphoproliferative responses to T cell mitogens become depressed in thermally injured guinea pigs at four days postburn and are maximally reduced by nine days postburn [4], experiments were performed during this time interval. Parenteral therapy of injured animals with indomethacin (10 mg/kg/day) significantly increased the proliferative response to mitogen at four days postburn but had no significant effect on this response at seven or nine days postburn ($P<0.05$; Table 1). Proliferative responses in injured animals treated with ibuprofen or

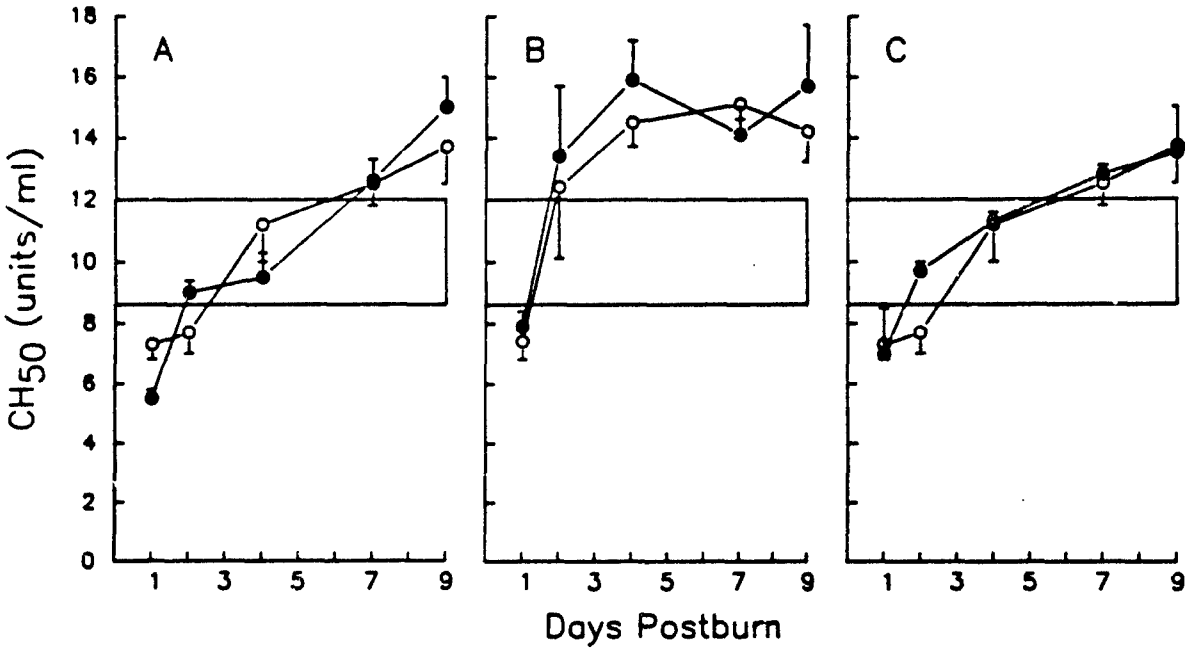

Figure 4. Hemolytic activity mediated by the alternative pathway in sera from thermally injured animals treated with NSAIDs (●) or placebo (○). Ten mg/kg indomethacin (A), 15 mg/kg ibuprofen (B), and 15 mg/kg piroxicam (C) were used in separate experiments, each having its own placebo group. Data are expressed as mean ± SEM. There were two to six animals per group. The open boxed areas show the range of hemolytic activity (mean ± 1SD) in sera from ten normal animals.

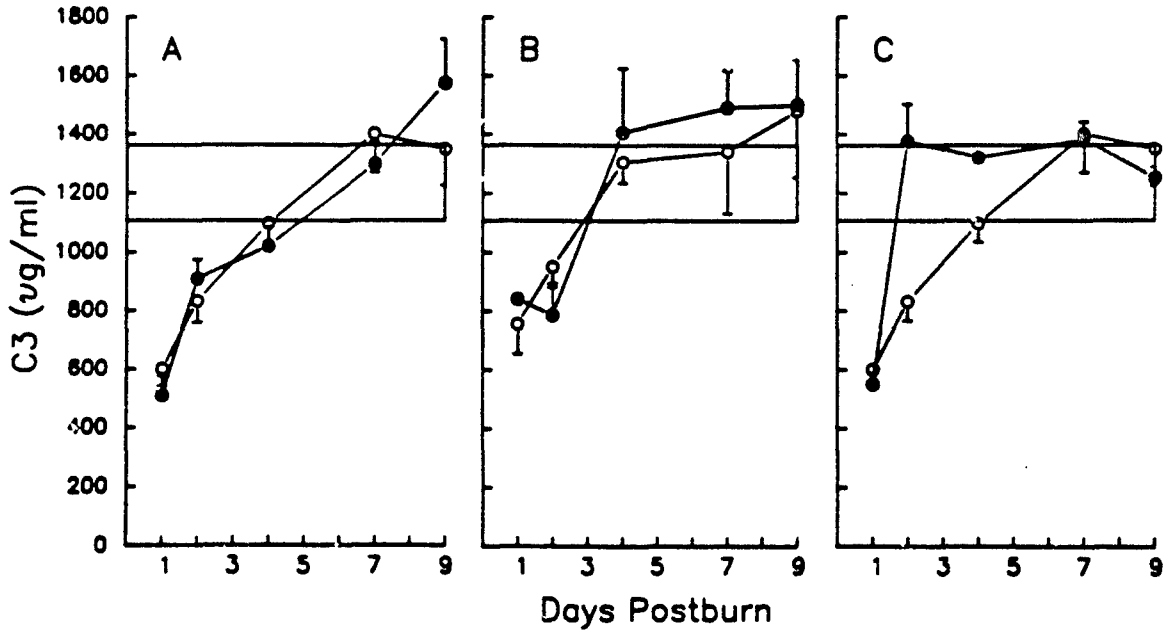

Figure 5. C3 concentrations in sera from thermally injured animals treated with NSAIDs (●) or placebo (○). Ten mg/kg indomethacin (A), 15 mg/kg ibuprofen (B), and 15 mg/kg piroxicam (C) were used in separate experiments, each having its own placebo group. Data are expressed as mean ± SEM. There were two to six animals per group. The open boxed areas show the range of C3 concentration (mean ± 1SD) in sera from ten normal animals.

Table 1. Effects of parenteral therapy with NSAIDs on the proliferative response of splenic lymphocytes to concanavalin A in thermally injured animals.

Treatment	Drug dose	[^{3}H]Thymidine incorporation*		
		Days postburn		
		4	7	9
	mg/kg/day	cpm x 10^{-3}		
Injured animals:				
Indomethacin	10	68.8 ± 7.6	21.8 ± 6.3	25.3 ± 6.0
	20	27.7 ± 11.5	13.7 ± 5.0	14.4 ± 8.6
Ibuprofen	15	36.0 ± 12.4	10.8 ± 2.6	24.1 ± 6.6
Piroxicam	15	20.0 ± 6.6	18.0 ± 7.5	11.6 ± 3.9
Placebo (once daily)		39.7 ± 9.8	14.3 ± 3.5	27.0 ± 7.3
Placebo (twice daily)		14.9 ± 8.2	14.6 ± 5.3	16.2 ± 5.8
Normal animals:				
No treatment		80.4 ± 7.7	63.0 ± 4.9	64.8 ± 6.8

* Data are expressed as mean ± SEM. There were 4 to 12 animals per group. Background responses in the absence of mitogen were less than 6,000 cpm.

piroxicam were not significantly different from those in injured animals treated with placebo.

To further investigate the effect of indomethacin on the lymphoproliferative response to mitogen, the dose of indomethacin administered to the injured animals was increased by twofold to 20 mg/kg/day. This dose of indomethacin had no significant effect on the proliferative response to mitogen in the injured animals (Table 1). These results and the failure to observe improvement in lymphoproliferative responses with ibuprofen and piroxicam therapy suggested that the early beneficial effect of the 10 mg/kg dose of indomethacin was not related solely to inhibition of the cyclooxygenase enzyme.

Effects of the NSAIDs on hematologic profile. Our previous studies have shown that the hematologic response to thermal injury in guinea pigs is characterized by thrombocytopenia, anemia, and early leukocytosis followed by leukopenia [4]. To determine the effects of the NSAIDs on these hematologic changes, complete blood counts and platelet counts were measured during nine days postburn in injured animals treated with NSAIDs or placebo. No significant differences in hematologic profile were observed in injured animals treated with indomethacin as compared with placebo, except platelet counts were significantly higher in indomethacin treated animals at seven days postburn ($P=0.02$; Table 2). Treatment of the injured animals with ibuprofen or piroxicam had no significant effect on the hematologic profile in the injured animals (results not shown).

Effects of the NSAIDs on resistance against burn wound infection. To determine if the enhancement of PMN bactericidal activity mediated by the NSAIDs led to an increase in the overall resistance against infection, the effects of the NSAIDs on survival following experimental burn wound infection with P. aeruginosa and P. mirabilis were determined. Each drug

Table 2. Effects of parenteral therapy with indomethacin on complete blood counts and platelet counts in thermally injured animals.*

Days postburn	Treatment	Platelets	Leukocytes	Erythrocytes	Hemoglobin	Hematocrit
		x $10^3/mm^3$	x $10^3/mm^3$	x $10^6/mm^3$	g/dl	%
Injured animals:						
1	Indomethacin	396 ± 39	5.3 ± 1.8	4.1 ± 0.2	10.9 ± 0.5	34 ± 1
	Placebo	383 ± 63	6.2 ± 1.4	3.8 ± 0.1	10.2 ± 0.4	31 ± 1
2	Indomethacin	500 ± 17	6.6 ± 0.9	4.1 ± 0.1	11.6 ± 0.5	34 ± 1
	Placebo	451 ± 50	4.1 ± 0.8	3.9 ± 0.2	10.7 ± 0.5	32 ± 1
4	Indomethacin	578 ± 88	5.8 ± 0.9	4.0 ± 0.1	11.0 ± 0.3	34 ± 1
	Placebo	412 ± 103	4.1 ± 0.7	3.8 ± 0.2	10.3 ± 0.4	31 ± 1
7	Indomethacin	554 ± 52	6.5 ± 1.7	4.2 ± 0.2	11.1 ± 0.4	33 ± 1
	Placebo	748 ± 45	3.9 ± 0.8	4.3 ± 0.2	11.7 ± 0.4	36 ± 1
9	Indomethacin	613 ± 119	3.5 ± 0.4	4.3 ± 0.1	12.0 ± 0.4	37 ± 1
	Placebo	469 ± 70	3.5 ± 0.7	4.2 ± 0.1	11.6 ± 0.3	36 ± 1
Normal animals:						
No treatment		606 ± 23	4.6 ± 0.6	5.2 ± 0.1	13.8 ± 0.4	41 ± 1

* Data are expressed as mean ± SEM. There were 5 to 10 animals in each group.

was tested in a separate experiment having its own placebo group. In initial experiments, the bacterial challenge was administered to the injured animals at 1.5 hr postburn, which was 1.5 hr prior to the initiation of therapy with the NSAIDs. Under these conditions, doses of 10 mg/kg of indomethacin and 15 mg/kg of ibuprofen and piroxicam did not influence either the extent of mortality or the time to death following infection with P. aeruginosa or P. mirabilis (Figures 6 and 7). Doses of 5 or 20 mg/kg of indomethacin also did not affect survival of infected animals (Figures 8 and 9). When the bacterial challenge was delayed to one day postburn to give the NSAIDs time to act before infection, survival was still unchanged in infected animals treated with NSAIDs as compared with placebo; results using indomethacin (10 mg/kg) and piroxicam (15 mg/kg) are shown in Figure 10. These results suggested that the enhancement of PMN bactericidal activity mediated by the NSAIDs was insufficient by itself to increase resistance against burn wound infection under the conditions of our experiments.

Discussion and Conclusions

We report in this communication the effects of parenteral therapy with indomethacin, ibuprofen, and piroxicam on key immunologic and hematologic alterations induced by thermal injury in a guinea pig model. The apy with the NSAIDs was initiated at 3 hr postburn to simulate conditions that could be potentially applied to the clinical care of thermally injured patients. NSAIDs were then administered daily for a period of nine days postburn. Preliminary studies confirmed that the doses of NSAIDs used in our study effectively inhibited prostaglandin and thromboxane production in wound fluid from the injured area, the major site of synthesis of these compounds. Thus, the NSAIDs acted as potent cyclooxygenase inhibitors under the conditions of our experiments.

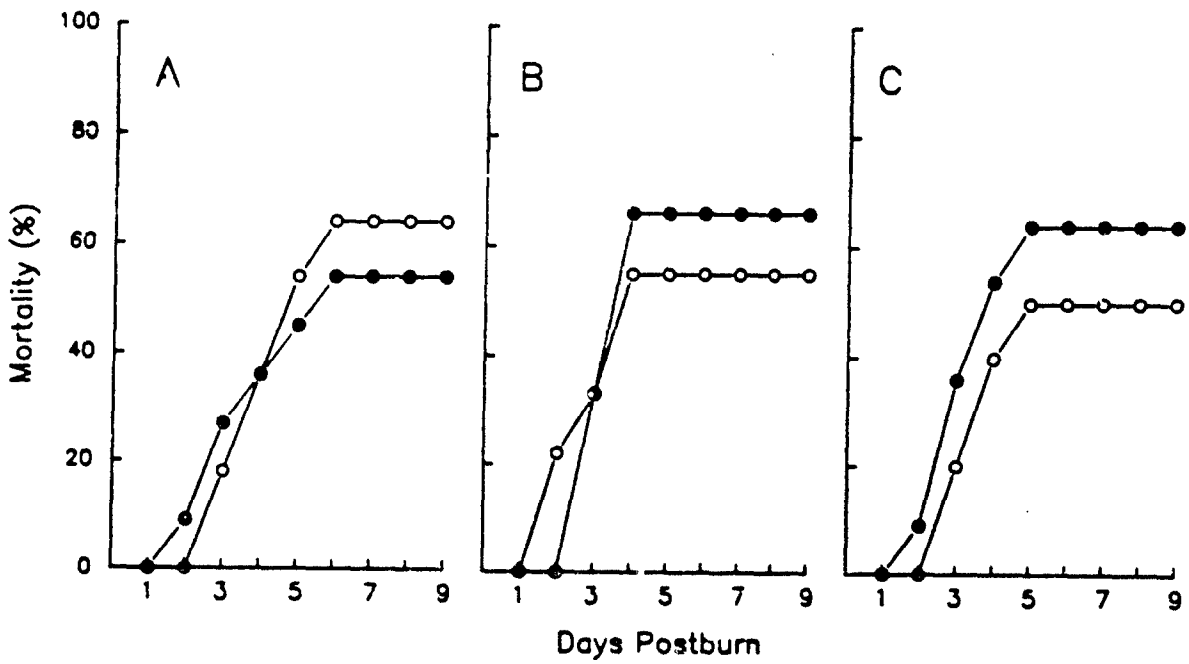

Figure 6. Mortality from burn wound infection with P. aeruginosa in thermally injured animals treated with NSAIDs (●) or placebo (○). Ten mg/kg indomethacin (A), 15 mg/kg ibuprofen (B), and 15 mg/kg piroxicam (C) were used in separate experiments, each having its own placebo group. The bacterial challenge was administered at 1.5 hr postburn. There were nine to eleven animals per group.

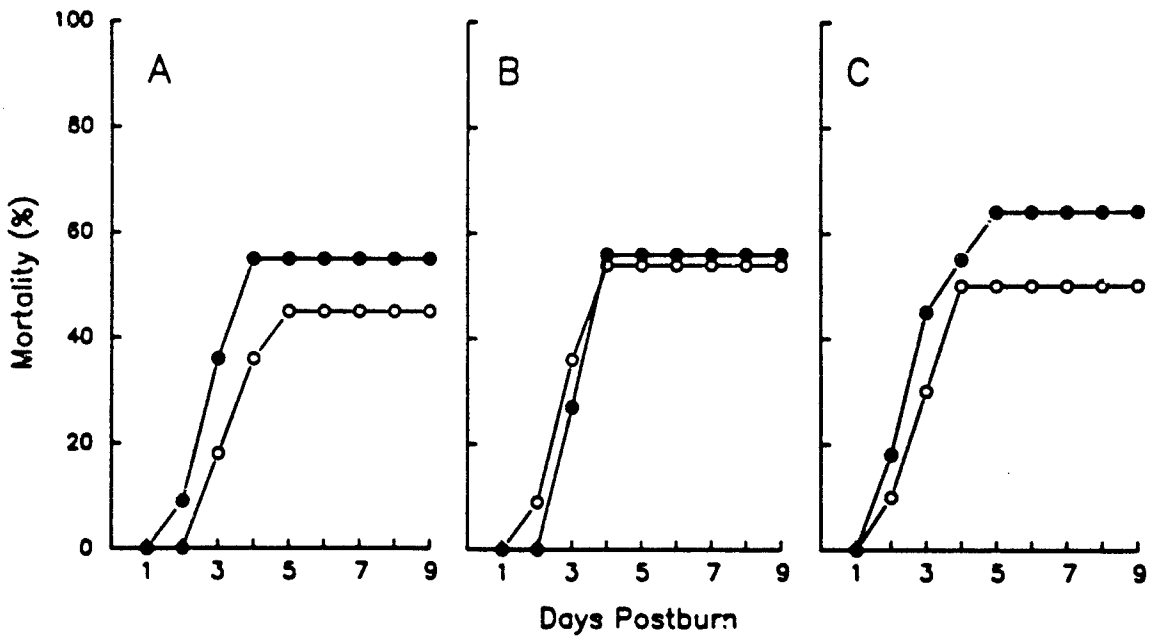

Figure 7. Mortality from burn wound infection with P. mirabilis in thermally injured animals treated with NSAIDs (●) or placebo (○). Ten mg/kg indomethacin (A), 15 mg/kg ibuprofen (B), and 15 mg/kg piroxicam (C) were used in separate experiments, each having its own placebo group. The bacterial challenge was administered at 1.5 hr postburn. There were ten to eleven animals per group.

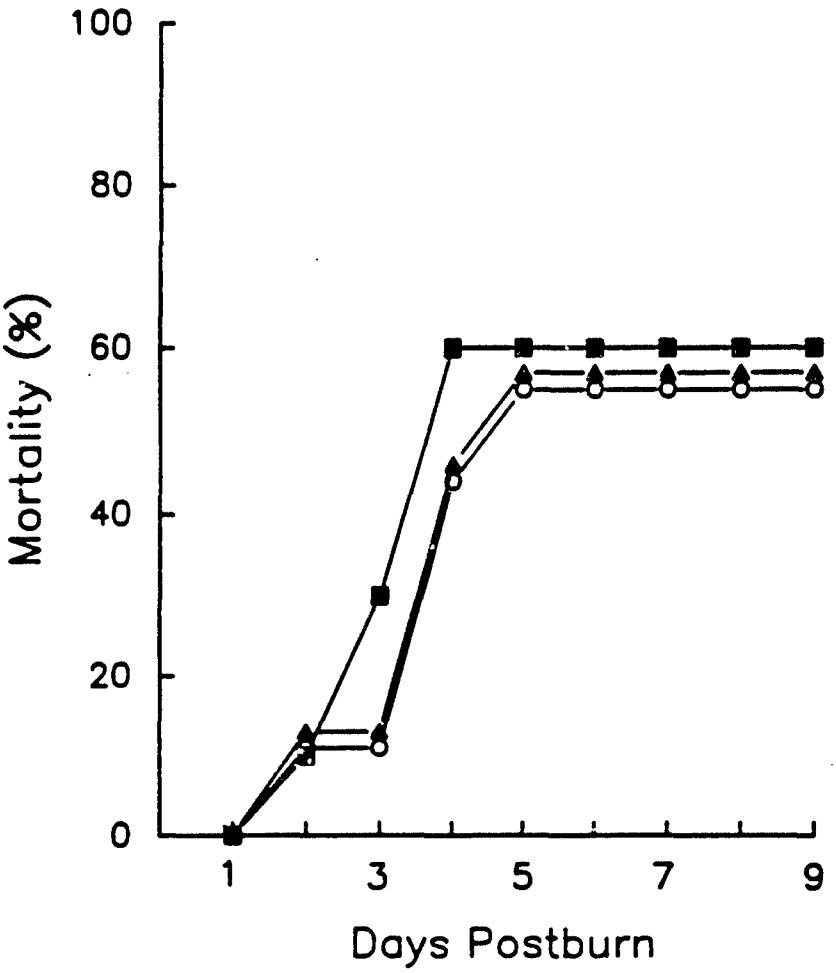

Figure 8. Mortality from burn wound infection with P. aeruginosa in thermally injured animals treated with 5 mg/kg indomethacin (■), 20 mg/kg indomethacin (▲), or placebo (○). The bacterial challenge was administered at 1.5 hr postburn. There were nine to ten animals per group.

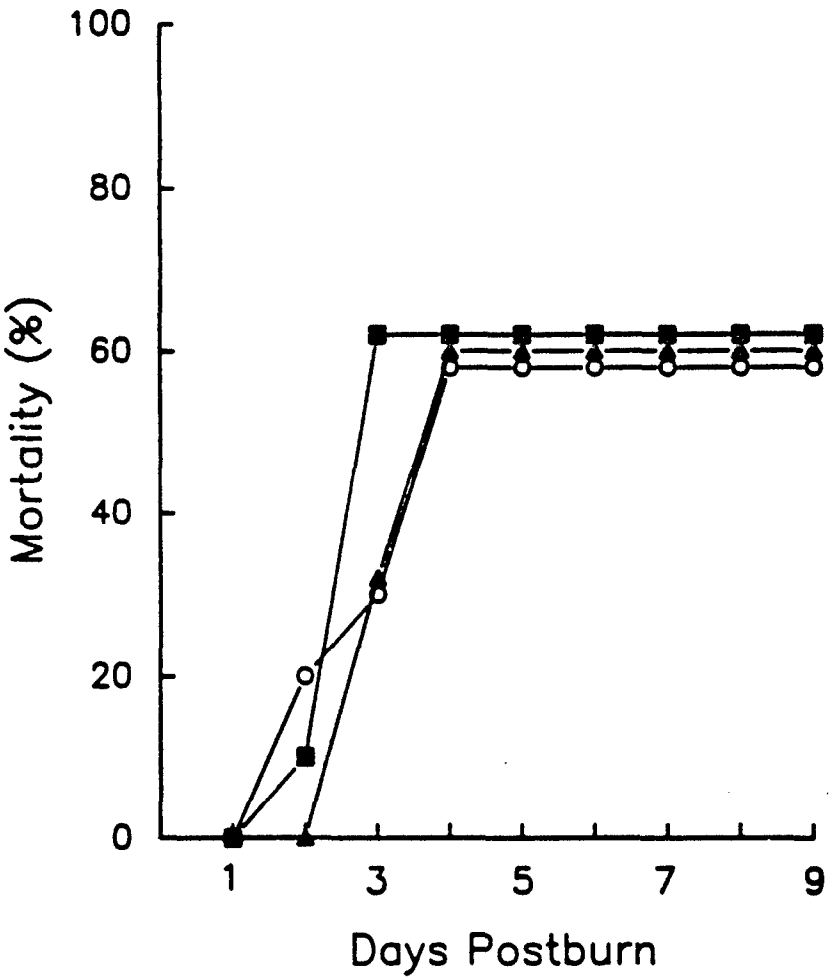

Figure 9. Mortality from burn wound infection with P. mirabilis in thermally injured animals treated with 5 mg/kg indomethacin (■), 20 mg/kg indomethacin (▲), or placebo (○). The bacterial challenge was administered at 1.5 hr postburn. There were ten animals per group.

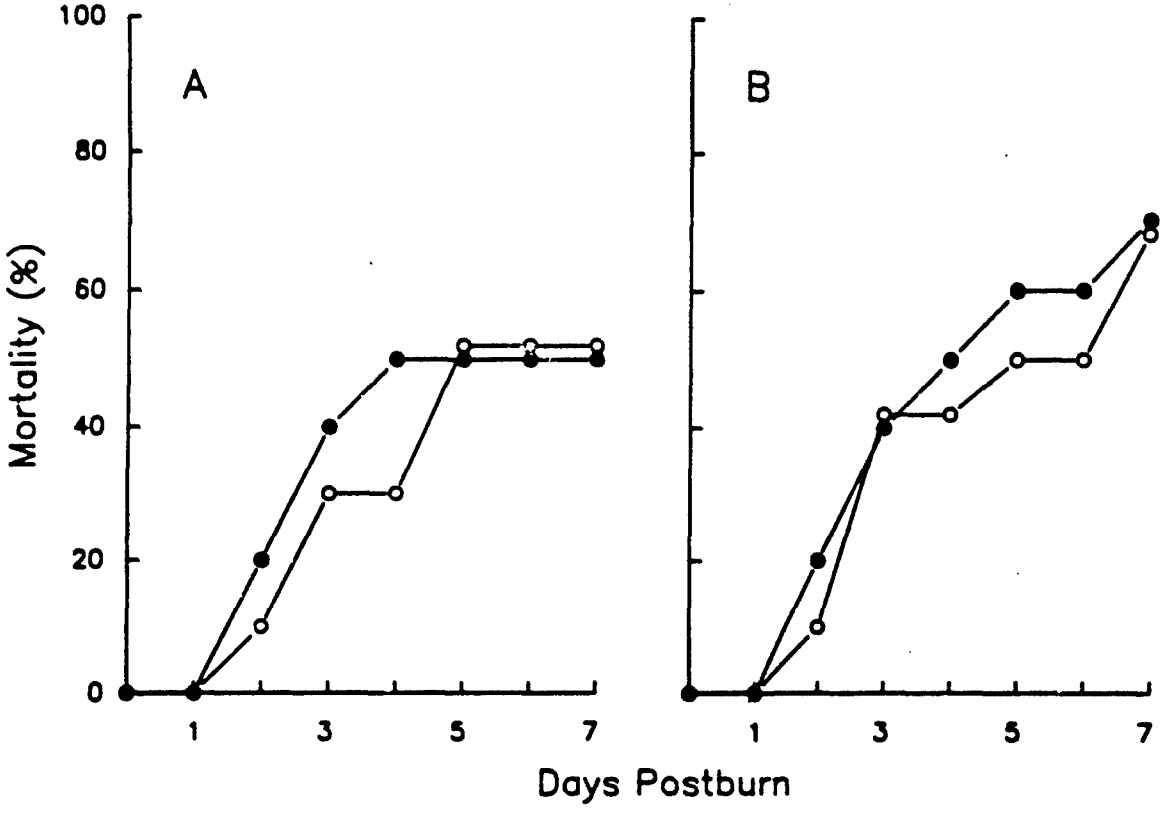

Figure 10. Mortality from burn wound infection with P. aeruginosa in thermally injured animals treated with NSAIDs (●) or placebo (○). Ten mg/kg indomethacin (A) and 15 mg/kg piroxicam (B) were used in separate experiments, each having its own placebo group. The bacterial challenge was administered at one day postburn. There were ten animals per group.

Initial studies focused on two major alterations in the first line of host defense associated with thermal injury, i.e., depression in the bactericidal activity of peripheral PMNs and systemic complement consumption. Previous studies from our laboratory had demonstrated that the occurrence of these alterations is temporally associated in the guinea pig model [4]. The results of the present investigation demonstrated that therapy with NSAIDs fully restored the bactericidal activity of PMNs from the thermally injured animals to normal without having a major effect on C3 levels or activity of the alternative pathway of the complement system in serum. It has been recently suggested that complement consumption, with formation of C5a, is the initial stimulus for activation and subsequent functional inactivation of PMNs in thermal injury [16]. Our observations suggest that NSAIDs interfere with this process at a stage occurring after complement consumption.

Prostaglandins of the E series are primary products of arachidonic acid metabolism via the cylooxygenase pathway in PMNs [19]. In addition, these prostaglandins are formed by other cells that are present at the site of thermal injury [20]. PGE_1 and PGE_2 have been shown to inhibit various functions of PMNs including aggregation, chemotaxis, superoxide production, and lysosomal enzyme release [21-26]. These prostaglandins may contribute to the functional inactivation of PMNs associated with thermal injury, and NSAIDs may restore PMN bactericidal activity to normal by inhibiting production of these compounds. NSAIDs may also have direct inhibitory effects on early steps in the activation of PMNs [27], and this may prevent the subsequent functional inactivation of these cells induced by thermal injury. It should be noted that NSAIDs, by inhibiting cyclooxygenase, can facilitate shunting of arachidonic acid metabolism through the lipoxygenase pathway [28,29]. Thus, it is possible that lipoxygenase products contri-

buted to the enhancement of bactericidal activity observed in our study.

Our investigation represents the first description, to our knowledge, of the preventability or reversibility of the bactericidal defect in PMNs associated with thermal injury. Alexander et al. have previously demonstrated some degree of improvement in the bactericidal activity of PMNs from thermally injured animals following therapy with Dazmegrel, a thromboxane synthetase inhibitor [30], and several immunomodulating agents [31, 32]. However, the effects were not as impressive as those observed in our study.

The effects of NSAIDs on the depression in lymphoproliferative response to T cell mitogen and on various hematologic changes induced by thermal injury were also determined in our investigation. Indomethacin at a dose of 10 mg/kg/day was found to increase the lymphoproliferative response to mitogen early after injury but not later during the course of recovery. Ibuprofen, piroxicam, and a twofold higher dose of indomethacin had no effect on this response. This observation suggests that the transient beneficial effect observed with the lower dose of indomethacin was related to a unique action of this drug manifested only at set concentrations. None of the NSAIDs affected the numbers of circulating leukocytes or platelets, or the degree of anemia in the injured animals.

Other studies have demonstrated greater effects of NSAIDs on cell mediated immune responses in thermally injured animals than those observed in our study. Hansbrough et al. [33,34] reported that parenteral therapy with indomethacin or ibuprofen increased delayed type hypersensitivity and restored the lymphocyte helper/suppressor ratio to a near normal level in thermally injured mice. Maghsudi and Miller [35] showed that indomethacin increased the secondary immune response to sheep erythrocytes in these animals. In both studies, the NSAIDs were administered in doses lower than

those used in our study, and no effort was made to determine if the drug regimens inhibited cyclooxygenase in the injured animals. It is therefore not possible to relate these findings to our own.

The effects of NSAIDs on survival following experimental burn wound infection with P. aeruginosa and P. mirabilis were also investigated in our study. The bacteria were spread over the surface of the burn wound to simulate conditions that occur naturally as a result of extensive colonization of the burn wound with bacteria. Infection caused by both organisms was invasive, and death occurred in approximately fifty percent of the animals by five days post infection. NSAIDs administered before or after infection had no effect on survival from infection under these conditions. This observation suggests that the marked enhancement of PMN bactericidal activity mediated by the NSAIDs was not by itself sufficent to increase the overall resistance of the animals to lethal infection. Cell mediated immunity may also be required for defense against infection caused by these virulent organisms, and our study failed to demonstrate an effect of NSAIDs on the depression in cell mediated immunity in the injured animals. Another explanation for our results is that NSAIDs may not prevent or reverse the impairment in chemotaxis of PMNs induced by injury, even though these drugs correct the bactericidal defect of these cells. The increased capacity of the PMNs to kill bacteria would be of limited value if the PMNs were unable to mobilize to sites of infection. Our continuing studies will address these various possibilities.

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